



The diet of adult and chick rock shags (*Phalacrocorax magellanicus*) inferred from combined pellet and stable isotope analyses

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Abstract

The current information about the diet composition of the rock shag (*Phalacrocorax magellanicus*) in the SW Atlantic coast comes mainly from conventional pellet or stomach content analysis from a few locations situated in northern Patagonia (Chubut Province, Argentina). In this work, we studied the diet of breeding rock shags over several years at a colony from southern Patagonia (Ría Deseado, Santa Cruz Province, Argentina) using a combined technique of conventional diet assessment (pellet analysis) and stable isotope analysis of carbon and nitrogen. Our results confirm the importance of benthic prey and the low inter-annual variability in the diet of the rock shag. These results coincide with previous research in relation to the exploitation of slow moving, predictable, but low-energy density prey. The stable isotope mixing models, which was informed with prior data obtained from pellet analysis, allowed for the detection of subtle differences between the diet of adults and chicks, consisting in the incorporation of higher proportions of cephalopods, an energy-rich prey, in the diet of chicks. By comparing our results with the diet of the red-legged cormorant, which breeds in sympatry in the Ría Deseado Estuary and whose diet composition is strongly pelagic, we suspect a certain level of trophic resource partitioning between these rock shag and red-legged cormorant.

Keywords Trophic ecology · Mixing model · Prior information · Cormorant · Seabird · Patagonia · Argentina

Introduction

Knowledge of seabirds' trophic ecology is fundamental to understand the ecosystem functioning and to assess potential impacts of oceanographic changes and anthropic activities on their populations (Weimerkirch et al. 2001; Suryan et al. 2006; Elliot et al. 2015). Furthermore, the diet of a specific seabird species can differ among colonies likely due to the effect of the community structure and the habitat characteristics of prey availability (Karnovsky et al. 2008; Chiaradia et al. 2012; Fernandez et al. 2019). The diet can also vary between years or breeding stages, in response to changes in food availability or individual demands (Bertellotti and

Yorio 1999; Weimerskirch et al. 2001; Carscadden et al. 2002; Ibarra et al. 2018).

The analysis of pellet casts (regurgitated indigestible remains of prey) has been widely used to study the diet of seabirds, as the sampling is simple and non-invasive (Barrett et al. 2007). Nevertheless, this approach can present some biases caused by the differential erosion rate of the prey remains that can over or under-represent certain prey types, and some secondary prey remnants can be retained (Barrett et al. 2007). This is why many authors (Barrett et al. 2007; Bond and Jones 2009; Ciancio et al. 2015) recommend the use of this conventional technique in combination with Stable Isotope Analysis (SIA) of carbon and nitrogen. Under the appropriate conditions, the SIA quantifies the relative importance of the main prey consumed with stable isotope mixing models (Parnell et al. 2010; Philips et al. 2014). One of the advantages of the SIA is that it provides integrated information on the diet assimilated over time and not at a punctual moment like conventional techniques (Barrett et al. 2007; Bond and Jones 2009). However, more specific dietary knowledge about potential prey is required to perform mixing models

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of a predator. That is why the SIA is an interesting tool to estimate prey proportions, especially when used in combination with conventional diet analysis. This becomes even more useful with the integration of prior information obtained from conventional diet in the SIA mixing models (Moore and Semmens 2008; Layman et al. 2012).

The rock shag (*Phalacrocorax magellanicus*) is endemic to the Atlantic and Pacific coasts of Patagonia, including the Falkland Islands (Malvinas) (Harrison and Peterson 1985; Johnsgard 1993; Yorio et al. 1998). In the Atlantic coast, a small population of around 7000 breeding pairs is distributed in 145 colonies, ranging between 42° 50' S to 54° 50' S (Yorio et al. 1998; Frere et al. 2005). Rock shags frequently nest together with imperial shags or red-legged cormorants, in mixed or adjacent colonies (Frere et al. 2005).

Like all shags and cormorants, rock shags are foot-propelled pursuit-divers; they feed mainly in shallow waters (< 10 m) near the coast (< 5 km) (Quintana et al. 2002). Frere et al. (2008) found that rock shags living in sympatry with red-legged cormorant (Ría Deseado, Santa Cruz) had significantly larger foraging ranges and trip durations than in allopatry (without red-legged cormorant; Caleta Malaspina, Chubut). They attributed these differences to possible interspecific competition mechanisms, forcing niche differentiation in areas of sympatry. Rock shag's diet has been studied through conventional analysis, and in only a few colonies from northern Patagonia (Chubut). It has been described as composed of a high proportion of benthic fish and invertebrates, including polychaetes, cephalopods and crustaceans and, to a lesser extent, demersal-pelagic fish (Malacalza et al. 1997; Punta et al. 2003; Bulgarella et al. 2008; Sapoznikow and Quintana 2009). Sapoznikow and Quintana (2009) found a very low seasonal and inter-annual variability in rock shag's diet, and suggest that its prey are relatively predictable and stable, although not abundant. These characteristics could help to explain its small population along the Patagonian coast (Quintana et al. 2002; Sapoznikow and Quintana 2005, 2009).

In this work, we studied the diet of breeding rock shags over several years at a colony from southern Patagonia using a combined technique of a conventional diet assessment (pellet analysis) and stable isotope analysis of carbon and nitrogen. Our objectives were to describe the diet of the rock shag at this colony and to assess the differences in diet composition among breeding stages (pellets) and between adults and chicks (SIA). Finally, we were also interested in discussing and contrasting the diet of this colony in which the rock shag breeds in sympatry with the red-legged cormorant with the diet of the rock shag from northern Patagonia in which it breeds in allopatry (without the red-legged cormorant).

Materials and methods

Study area

The Ría Deseado Estuary is found in southern Patagonia, near the town of Puerto Deseado, on the northern coast of the Santa Cruz Province in Argentina (47° 45' S, 65° 53' W). This estuary, or ria, is a long (> 40 km), narrow inlet formed by the partial submergence of a river valley, and belongs to the protected area *Reserva Provincial Ría Deseado*. The fieldwork was carried out at Isla Elena (47° 45' S, 65° 56' W), an island situated 5 km from the entrance of the ria, hosting the only breeding colony of rock shags from the Ría Deseado (124 breeding pairs, Gandini and Frere 1998; Frere et al. 2005).

Conventional diet sampling

Pellets were collected at Isla Elena during four breeding seasons (2009, 2011, 2012 and 2013) by fixing collecting bags (2 × 0.7 × 0.5 m) on the cliffs, underneath two groups of three to five nests each. The bags containing the pellets were emptied every 1 to 2 weeks between November and February. The pellets were collected during three breeding stages: incubation (approximately early-November to mid-December), early chick-rearing (chicks up to 4 weeks old; mid-December to mid-January), and late chick-rearing (chicks between approximately 4 weeks old and fledglings; mid-January to end-February). The pellets were analysed with a binocular microscope and hard prey remains were used to quantify and identify prey at the lowest taxonomic level possible. Identification was carried out by using our own collections and available literature and catalogues (Lombarte et al. 1991; Boschi et al. 1992; Gosztonyi and Kuba 1996; Pineda et al. 1996; Piacentino 1999; Volpedo and Echeverría 2000; Tombari et al. 2010). The frequency of occurrence (%FO) and the number of occurrence (%N) were calculated for all prey items, and expressed as percentages. Published or our own allometric regressions were used to estimate the average total length (TL) and wet weight (W) of different prey types (Pineda et al. 1996; Koen-Alonso et al. 2000). The length of fish otoliths and cephalopod mandibles from the 2009 season pellets were used for TL and W calculations. Finally, the Shannon–Weaver diversity index was calculated for the whole time period (all years together) and for each separate year (Tramer 1969).

SIA sample collection and processing

Whole blood samples of adult and chick rock shags were collected during three consecutive breeding seasons (2011 to 2013) at Isla Elena (overall $N = 53$) for SIA. Adults were

captured from their nests during late-incubation to early chick-rearing stage (mid-December), corresponding to the period of time when they were less prone to fly away (Frere et al. 2002). Each manipulation lasted less than 5 min, and on release, birds flew directly to the water and returned to their nests shortly afterwards. The 2- to-4 week old chicks were captured from their nests in early January, and put back promptly after manipulation. Approximately, 0.5 mL of blood was extracted from the brachial vein of adults and chicks, and preserved in 70% ethanol before processing in the laboratory (Hobson et al. 1997).

Samples of the main prey sources for SIA were collected from 2011 to 2013 spring–summer seasons. Four potential prey sources were chosen according to our preliminary dietary results and unpublished information of the same area (Gandini and Frere, unpublished data). These prey were two demersal-benthic fish groups: the rock cods (*Patagonotothen cornucola*) and the eelpouts (zoarcidae fish), and two different cephalopods: the benthic octopus (*Enteroctopus megaloctyathus*, known as red octopus) and the demersal-pelagic squid (*Doryteuthis gahi*, known as Patagonian squid). Fish samples were collected under intertidal rocks, squid samples were found from regurgitates in the collecting bags or around nests, and octopus' samples were obtained from local fishermen. All prey samples were identified using the available literature and catalogues (Menni et al. 1984; Pineda et al. 1998; Bovcon et al. 2007) and were stored frozen until processing in the laboratory.

The samples were dried at 60 °C for > 24 h for whole blood, and for > 48 h for fish muscle and cephalopod mantle samples, and ground to a fine, homogenized powder. Carbon and nitrogen isotope ratios were measured in the Center for Stable Isotopes at the University of New Mexico, USA, by Elemental Analyser Continuous Flow Isotope Ratio Mass Spectrometry using a Costech ECS 4010 Elemental Analyser coupled to a Thermo Fisher Scientific Delta V Advantage mass spectrometer via a CONFLO IV interface. Isotope ratios were reported using the standard delta (δ) notation relative to AIR and Vienna Pee Dee Belemnite (V-PDB), respectively, and expressed in units per thousand (‰) as follows: $\delta = (R_{\text{sample}}/R_{\text{standard}} - 1)$, where R_{sample} and R_{standard} are the molar ratios of the heavy to light isotopes ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) of the sample and standard, respectively. Average analytic precision based on routine analysis of a laboratory protein standard was < than 0.1‰ (1 σ). The laboratory standard was calibrated against IAEA-N-1, IAEA-N-2, USGS 42 and USGS 43 for nitrogen and NBS 21, NBS 22 and USGS 24, USGS 42 and USGS 43 for carbon.

Posterior inspection of C/N mass ratio showed that the eelpout (zoarcidae fish) presented several C/N values > 4, indicating possible bias in $\delta^{13}\text{C}$ due to high lipid content (Post et al. 2007; Bond and Jones 2009). Therefore, $\delta^{13}\text{C}$

values of the eelpout were corrected with a mathematical model according to Logan et al. (2008). Rock cod and Patagonian squid SIA values used in this study were previously published by Morgenthaler et al. (2016).

SIA mixing models

The relative contribution of the potential prey to the diet of the shags was estimated using Bayesian mixing models from the 'simmr' package (Parnell 2016) in R 3.6.0 (R Core Team 2019). Due to inter-annual differences of shag and prey isotopic data, models were run for each year separately, with adults and chicks considered as two different groups. The prey sources data used for each model (year) were from the same year as the consumers' data, when available (see Table 2). Two different models were run for each year: (1) an initial model, with no prior information and (2) an informed model with the following priors obtained from conventional diet: rock cod: 0.4; eelpout: 0.25; octopus: 0.25; squid: 0.1.

Up to the researchers' knowledge, no diet to whole blood discrimination factor, a key parameter for stable isotope mixing models, has been experimentally determined for the rock shag. Only an experimentally determined value is known for a species of shag/cormorant: the double-crested cormorant (*Phalacrocorax auritus*) which was fed with catfish (*Ictalurus punctatus*), a freshwater farm fish (Craig et al. 2015). In our study, the whole blood discrimination factor the Magellanic penguin (*Spheniscus magellanicus*) a seabird species that coexists with the rock shag in the Ría Deseado, was used (Ciancio et al. 2016). Although this factor was obtained from a taxonomically different bird family, the feeding trials were made with anchovy (*Engraulis anchoita*), a prey found in the Patagonian Sea which is ecologically and isotopically more similar to the prey of rock shag than the freshwater farm-raised catfish fed to the double-crested cormorant. The Δ discrimination factor values used were: +2.31‰ for $\Delta^{15}\text{N}$ and +0.41‰ for $\Delta^{13}\text{C}$ (Ciancio et al. 2016); a standard deviation of ± 1 ‰ was included to take into account the uncertainty due to possible differences in the discrimination factors between penguins and shags (Votier et al. 2010). The simulation method for inspection of mixing polygons from Smith et al. (2013) confirmed that the consumer data were situated within the 95% mixing regions for each model (probability ranges for each model: 2011: 0.55 to 0.69, 2012: 0.35 to 0.65, 2013: 0.23 to 0.66).

Statistical analyses

For conventional diet, multivariate similarity analyses (ANOSIM) using the R 'vegan' package were used to test for differences in the biomass estimates of the main prey types among years and among breeding stages (Oksanen et al. 2016). The isotopic centroid positions were examined

using nested linear models and residual permutation procedures (Turner et al. 2010). Centroid locations were compared among each pair of years (adults and chicks together), and between adults and chicks within each year, and were

considered different if the Euclidean Distance (ED) between centroid locations was significantly greater than zero (Turner et al. 2010).

Table 1 Percentages of prey in the diet of breeding rock shag at Isla Elena

	2009		2011		2012		2013	
	%N	%FO	%N	%FO	%N	%FO	%N	%FO
<i>N</i> prey items— <i>N</i> pellets	1086	10	1187	20	1162	20	870	20
TELEOST FISHES	30.8	90	43.4	100	40.1	95	62.3	100
<i>Patagonotothen</i> spp. (DB)	8.7	90	15.7	100	16.9	85	25.9	95
Zorcidae (DB)	0.2	20	3.7	50	5.2	70	15.4	90
<i>Phucocoetes latitans</i> (DB)	0.0	0	1.5	25	1.8	40	8.4	70
<i>Iluocoetes elongatus</i> (DB)	0.1	10	0.9	15	0.2	10	0.7	20
<i>Austrolycus laticinctus</i> (DB)	0.0	0	0.2	5	0.3	10	0.8	20
<i>Dadyanos insignis</i> (DB)	0.0	0	0.1	5	0.1	5	1.8	25
Unidentified zorcidiae	0.1	10	1.0	30	2.7	55	3.9	30
<i>Agonopsis chilensis</i> (DB)	0.9	30	1.7	35	1.4	30	1.5	35
<i>Cottoperca gobio</i> (DB)	0.0	0	0.1	5	0.3	5	0.1	5
<i>Salilota australis</i> (DP)	0.0	0	0.1	5	0.0	0	0.1	5
<i>Paranotothenia magellanica</i> (DB-DP)	0.0	0	0.2	10	0.0	0	0.0	0
<i>Eleginops maclovinus</i> (DB)	0.0	0	0.0	0	0.1	5	0.0	0
<i>Odontesthes</i> spp.(DP)	0.0	0	0.1	5	0.0	0	0.0	0
Unidentified fish	20.9	90	21.9	90	16.2	80	19.3	75
CEPHALOPODS	1.0	50	1.3	40	1.0	25	1.1	30
<i>Enteroctopus megalocyathus</i> (DB)	1.0	50	0.8	30	1.0	20	0.5	15
<i>Doryteuthis gahi</i> (DP)	0.0	0	0.4	20	0.0	0	0.7	20
CRUSTACEANS	1.7	80	3.7	95	2.0	75	2.1	70
Decapoda	0.6	60	2.5	70	1.4	50	1.4	60
<i>Eurypodius latreilli</i> (B)	0.3	20	0.8	25	0.0	0	0.2	10
<i>Peltarion spinosulum</i> (B)	0.0	0	0.1	5	0.3	5	0.1	5
<i>Halicarcinus planatus</i> (B)	0.0	0	0.0	0	0.0	0	0.1	5
<i>Betaeus truncatus</i> (DB)	0.0	0	0.0	0	0.9	40	0.2	10
Callianassidae (B)	0.0	0	0.1	5	0.0	0	0.0	0
Unidentified decapoda	0.4	40	1.6	50	0.3	10	0.7	25
Isopoda	0.7	40	0.9	40	0.6	30	0.3	15
Stomatopoda	0.0	0	0.0	0	0.0	0	0.1	5
Amphipoda	0.0	0	0.1	5	0.0	0	0.0	0
Unidentified crustaceans	0.3	30	0.2	10	0.0	0	0.2	10
POLYCHAETES	66.5	100	51.3	100	56.7	95	33.8	95
Polynoidae (B)	37.7	90	23.8	90	18.8	90	21.4	85
Nereididae (B)	26.2	90	25.3	95	37.3	85	11.3	80
Eunicidae (B)	1.9	30	1.6	30	0.6	30	1.1	35
Unidentified polychaetes	0.7	20	0.6	5	0.1	5	0.0	0
GASTEROPODS (B)	0.1	10	0.2	10	0.1	5	0.7	30
ASCIDIANS (B)	0.0	0	0.1	5	0.1	5	0.0	0
ALGAE (B)		70		75		45		45

Number of individual prey (*n*), percent number (%N), percent frequency of occurrence (%FO) of prey obtained from 70 pellets. The ecological group of each prey is shown in parentheses (*P* pelagic, *B* benthic, *DP* demersal-pelagic, *DB* demersal-benthic)

Results

Conventional diet

Out of 70 analysed pellets, 26 different prey items belonging to seven different taxa (teleost fishes, cephalopods, crustaceans, polychaetes, gasteropods, ascidians and algae) were identified (Table 1). Overall prey diversity was high (Shannon–Weaver Index: 1.43) and did not vary much among years (2009: 1.16, 2011: 1.45, 2012: 1.35 and 2013: 1.57) and among breeding stages (mean value of all 4 years: incubation: 1.34, early chick rearing: 1.58 and late chick rearing: 1.38). The dietary composition, considering the biomass estimates of the main prey types, showed a high level of overlap and no significant differences among breeding stages ($R=0.031$, $p=0.175$), and a high level of overlap but significant differences among years ($R=0.050$, $p=0.044$).

The most important prey, based on the biomass estimates, were the rock cods (*Patagonotothen* spp.), followed by eelpouts (zoarcidae fishes) and by the red octopus (*Enteroctopus megalocyathus*) (Fig. 1). This later prey showed higher estimates during the early chick rearing stage (Fig. 1). The most frequent prey (highest frequency of occurrence) were

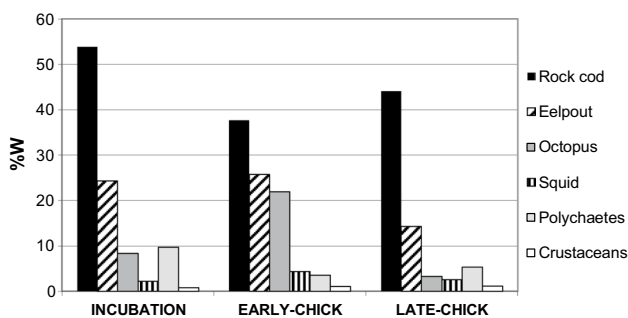


Fig. 1 Percent estimated wet weight (%W) of the main prey for each breeding stage in the diet of rock shag based on pellet analysis. The prey presented in the figure are: Rock cod, *Patagonotothen* spp. in black; Eelpout (zoarcidae fishes) with black-white diagonal lines; Red octopus, *Enteroctopus megalocyathus*, in dark grey; Patagonian squid, *Doryteuthis gahi*, with black-white vertical lines; Polychaetes worms in light grey and Crustaceans in white. Values are shown for each of the three breeding stages: incubation, early chick-rearing and late chick-rearing from Isla Elena colony, all years together (number of pellets analyzed: N incubation=31, N early- chick=28, N late chick=12)

the rock cod and the polychaete worms (Table 1). Polychaetes were also the most numerically abundant prey. Although their individual size and weight is small, their overall estimated biomass (%W) during the incubation stage accounted for 9.7% (Fig. 1). All the main prey of the rock shag are considered demersal-benthic or benthic, except the demersal–pelagic Patagonian squid (*Doryteuthis gahi*) (mean W%: 3.1%).

Stable isotope values and diet estimated from the mixing models

The stable isotope values of rock shag whole blood ranged between -17.1 and -13.7‰ for $\delta^{13}\text{C}$, and between $+17.8$ and $+19.6\text{‰}$ for $\delta^{15}\text{N}$ (Table 2). Although the distances of the mean isotopic positions (adults and chicks together) among all pairs of years were small ($\text{ED}=0.3\text{--}0.5\text{‰}$), the positions were significantly different between 2011 and 2012 ($\text{ED}=0.5\text{‰}$, $p=0.05$). The mean isotopic position of adults and chicks differed significantly within years in 2011 and 2012, with adults presenting higher $\delta^{13}\text{C}$ values than chicks (Table 2, Fig. 2). The mean isotopic position of the rock cod (*Patagonotothen cornucola*), the main prey of shags, differed significantly between 2011 and 2012 ($\text{ED}=2.2\text{‰}$, $p=0.001$), and between 2011 and 2013 ($\text{ED}=1.5\text{‰}$, $p=0.03$), but not between 2012 and 2013 ($\text{ED}=0.7\text{‰}$, $p=0.3$; Table 3).

The results of the initial mixing models (model 1) of adult shags showed similar proportions of the three benthic prey (rock cod, eelpout and octopus) for 2011, 2012 and 2013, which ranged between 21 and 33% each, and lower proportions of the pelagic prey (squid: 13–18%). All proportion estimates showed high credibility intervals (Fig. 3). The models for the chicks showed proportions for all four prey varying between 17 and 35%, with high credibility intervals, and variable order of importance of prey among years (Fig. 3). The results of the informed mixing models (model 2) reduced the credibility intervals in all proportion of prey estimates, restricting the results to a range of proportions more similar to those obtained with conventional diet analysis, with the rock cod found as the main prey in all the models (Fig. 4). Despite the fact that the adult and chick models

Table 2 Stable isotope values of adults and chicks of rock shag

Year	<i>n</i>	$\delta^{13}\text{C}$ (s.d.)	$\delta^{15}\text{N}$ (s.d.)	<i>n</i>	$\delta^{13}\text{C}$ (s.d.)	$\delta^{15}\text{N}$ (s.d.)	ED
2011	8	-15.7 (0.5)	19.2 (0.3)	9	-16.2 (0.5)	18.8 (0.4)	0.63*
2012	6	-15.1 (0.3)	18.7 (0.5)	10	-16.2 (0.6)	18.4 (0.4)	1.12*
2013	10	-15.6 (1.1)	19.0 (0.3)	10	-15.6 (0.4)	18.5 (0.2)	0.48*

Data are presented as means with standard deviation (in parentheses). Euclidean distances (ED) between centroids of adults and chicks are shown for each year and the star “*” indicates significant difference in their centroid position

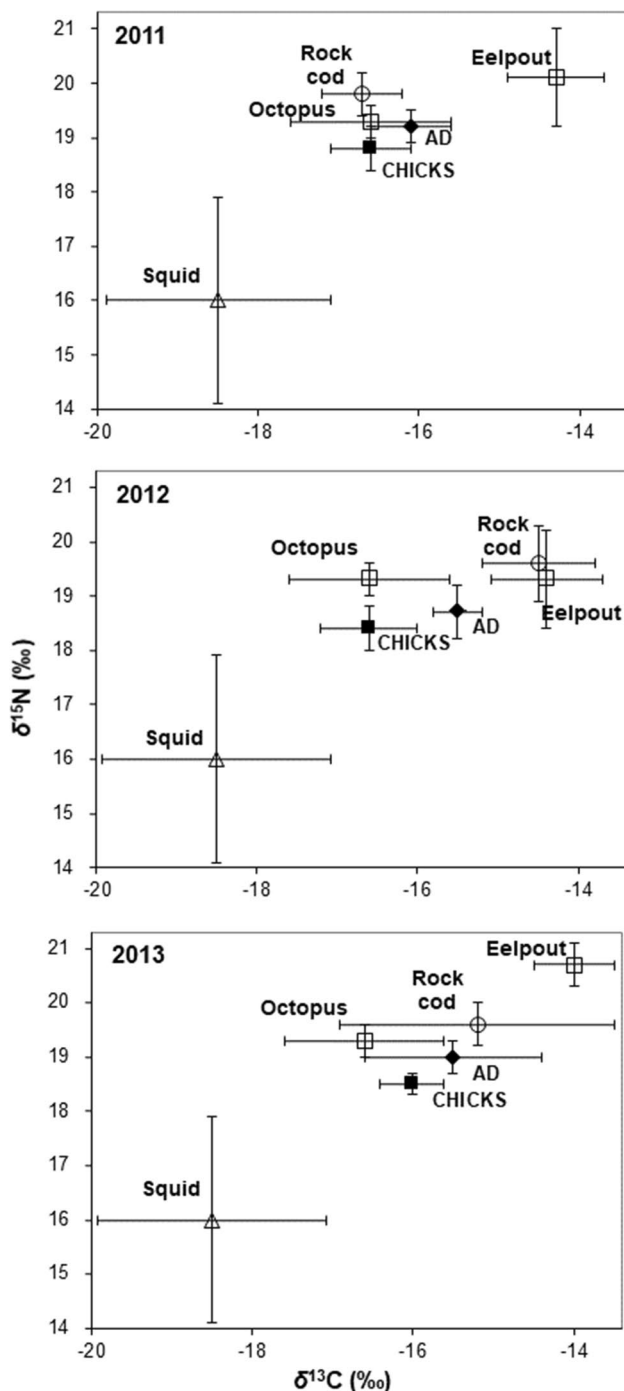


Fig. 2 Stable isotope mixing diagram of adults (AD) and chicks (CHICKS) of rock shag (black) from Isla Elena and their potential prey (white), presented by year. Values are mean \pm s.d.. Stable isotope values of the prey were corrected by adding the Diet Tissue Discrimination Factor (see methods). Prey items: Rock cod, *Patagonotothen* spp., Eelpout (zoarcidae fishes), Red octopus, *Enteroctopus megalocyathus*, and Patagonian squid, *Doryteuthis gahi*

Table 3 Stable isotope values of main prey of rock shag from Ría Deseado

Prey year	<i>n</i>	$\delta^{13}\text{C}$ (s.d.)	$\delta^{15}\text{N}$ (s.d.)
<i>Patagonotothen cornucola</i> (Rock cod) ⁺			
2011	3	-16.7 (0.5)	17.5 (0.4)
2012	5	-14.5 (0.6)	17.6 (0.7)
2013	5	-15.2 (1.7)	17.3 (0.4)
Zoarcidae (Eelpout)			
Years combined	8	-14.3 (0.6)	17.8 (0.9)
2012	5	-14.4 (0.7)	17.5 (0.9)
2013	3	-14.0 (0.5)	18.4 (0.4)
<i>Doryteuthis gahi</i> (Patagonian squid) ⁺			
Years combined	7	-18.5 (1.4)	13.7 (1.9)
<i>Enteroctopus megalocyathus</i> (Red octopus)			
Years combined	4	-16.6 (1.0)	17.0 (0.3)

Data are presented as means with standard deviation (in parentheses). All data are corresponding to the mentioned year, but *Doryteuthis gahi* ($N_{2012}=5$, $N_{2013}=1$), *Enteroctopus megalocyathus* ($N_{2013}=4$) and Zoarcidae fishes used in 2011 models ($N_{2012}=5$, $N_{2013}=3$)

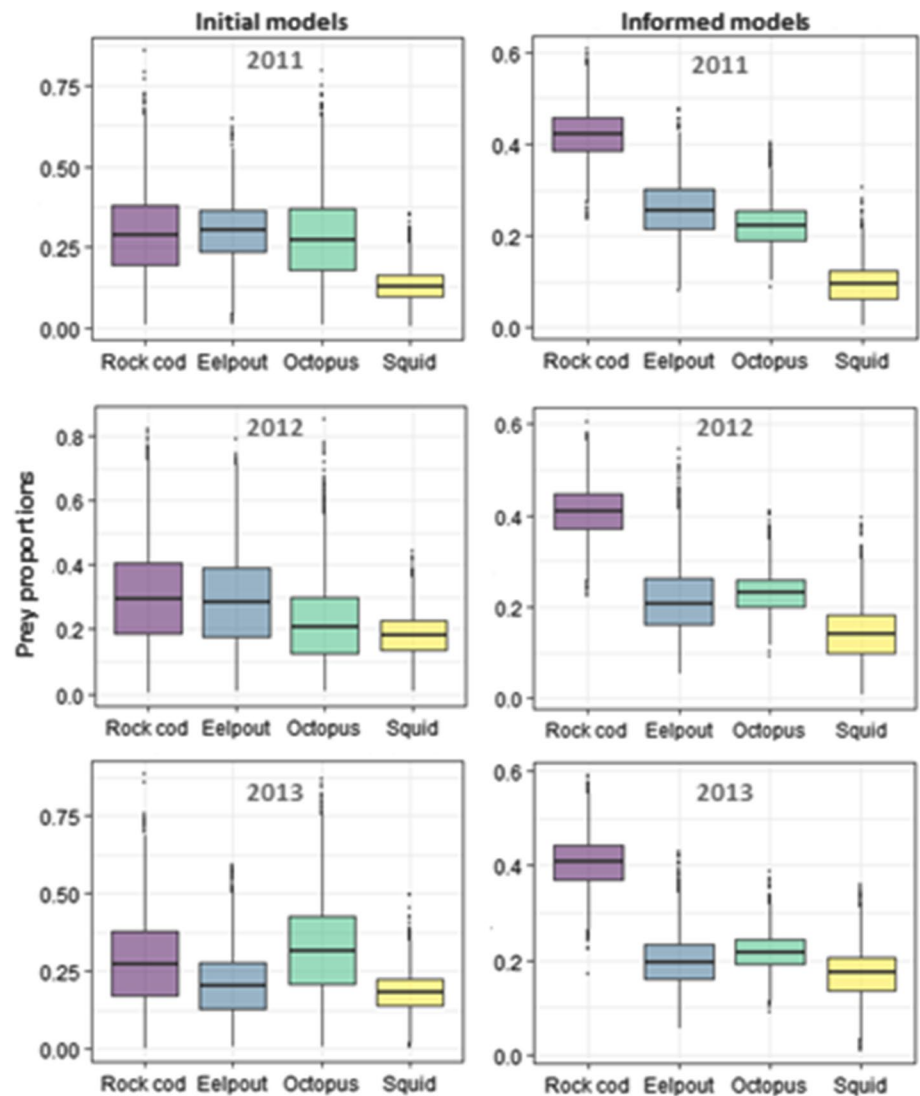
Data marked with “+” were originally published in Morgenthaler et al. 2016

were informed with identical priors, chicks showed higher proportions of squid than adults for every year (Fig. 4).

Discussion

Our diet results from southern Patagonia are in accordance with previous diet studies of Rock shag from northern Patagonia (Malacalza et al. 1997; Punta et al. 2003; Bulgarella et al. 2008; Sapoznikow and Quintana 2009) showing that benthic species of fish are the most frequent and/or abundant prey. Rock cod (*Patagonotothen* spp.) is the most abundant prey for both adult and chick shags in our study, followed by another benthic fish group, the eelpout (zoarcidae). Both rock cods and eelpouts live in similar habitat, and are usually associated with rocky bottoms and holdfasts of kelp (*Macrocystis pyrifera*) forest (Vanella et al. 2007). The benthic rock cod is considered a low-energy content prey, contrary to pelagic fish (Ciancio et al. 2007; Fernández et al. 2009). Punta et al. (2003) and Malacalza et al. (1997) found some pelagic or demersal fish species (*Engraulis anchoita* and *Merluccius hubbsi* respectively) in the diet of rock shags, although in much smaller proportions than benthic

Fig. 3 Estimated prey contributions to the diet of adult rock shags obtained from initial (left) and informed (right) stable isotope mixing models. Box plots display the range between 25 and 75% credibility quantiles, with error bars extending to the maximum and minimal values (97.5% and 2.5%, respectively), and the median represented by the bold line. Prior data of informed models: *Rock cod*: 0.4; *Eelpout*: 0.25; *Octopus*: 0.25; *Squid*: 0.1



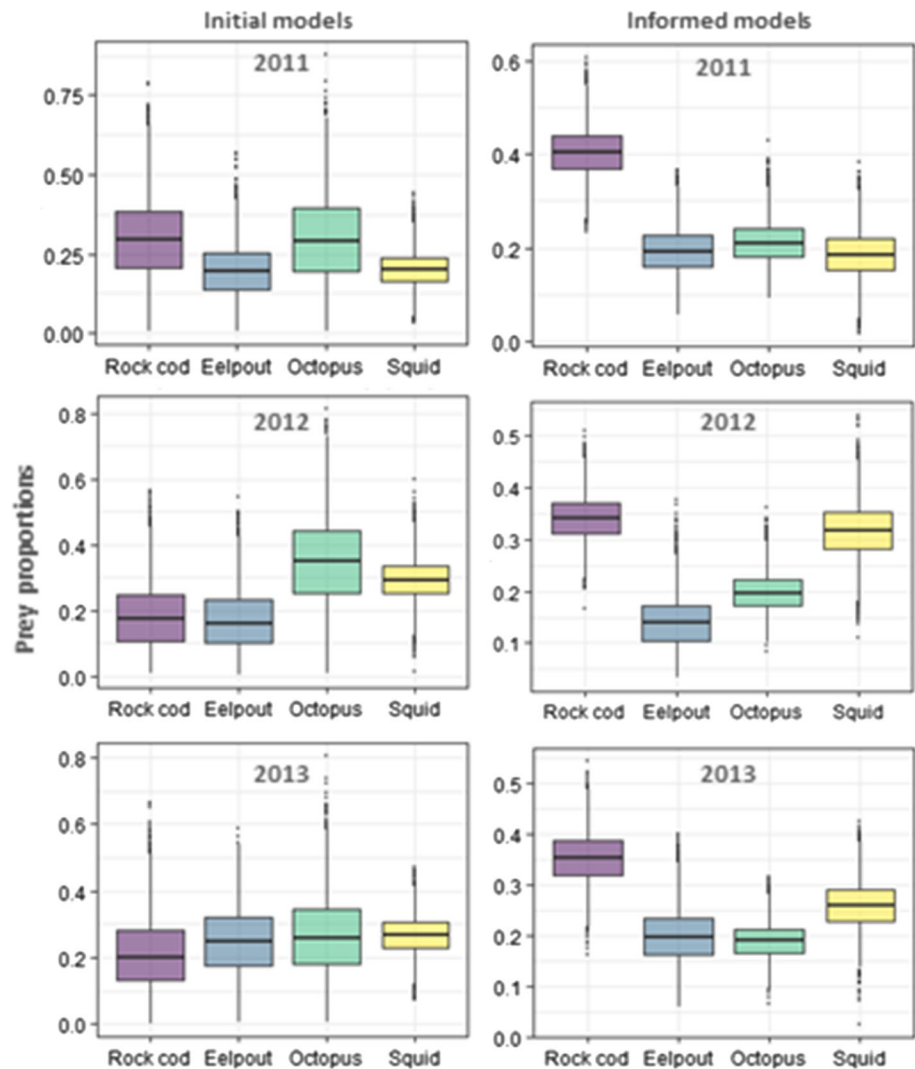
species. No such prey are found in our study, despite the presence of pelagic or demersal-pelagic small fish (*Sprattus fuegensis* and *Odontesthes* spp.) in the Ría Deseado (Frere et al. 2002; Nasca et al. 2004; Millones et al. 2005). Nevertheless, the benthic eelpouts might be considered a higher energy content prey than benthic rock cods due to the high lipid content found in this study ($C:N > 4$), and could therefore play an important role in the diet of breeding rock shags at Ría Deseado.

The diet of the rock shag at Ría Deseado is very diverse and includes several invertebrate groups. The most frequent and numerically abundant (%FO and %N) are the polychaete worms, which, despite their small size, contribute

to a non-negligible percentage of biomass (%W: 6.2). Although some authors consider the polychaetes as secondary prey (Casaux et al. 1995), Sapoznikow and Quintana 2009 considered them as primary prey because of their high frequency and abundance (some pellets only contained polychaetes' mandibles and chaeta), which is similar to our study. Polychaetes are interesting from an energetic perspective because of their energy content, comparable or superior to that of benthic fish (Ciancio et al 2007; Sapoznikow and Quintana 2009).

The most abundant (%W and mixing models proportions) invertebrate group are the cephalopods, composed of one octopus and one squid species. Interestingly, the mixing

Fig. 4 Estimated prey contributions to the diet of chick rock shags obtained from initial (left) and informed (right) stable isotope mixing models. Box plots display the range between 25 and 75% credibility quantiles, with error bars extending to the maximum and minimal values (97.5% and 2.5%, respectively), and the median represented by the bold line. Prior data of informed models: *Rock cod*: 0.4; *Eelpout*: 0.25; *Octopus*: 0.25; *Squid*: 0.1



models allowed to determine that cephalopods proportion estimates were higher in chicks than in adults. Seabirds sometimes feed their chicks with different prey items from what the adults eat for themselves (Davoren and Burger 1999). The incorporation of cephalopods during chick rearing period is likely to be an interesting strategy from an energetic perspective, as both the octopus and the squid have higher energy content than benthic fishes, like rock cods (Ciancio et al 2007; Sapoznikow and Quintana 2009). The Patagonian squid is the only demersal-pelagic prey found in the diet of the rock shag. It is an abundant species on the coast of northern Santa Cruz, and it is often preyed upon by other colonial seabird nesting in the Ría Deseado estuary (Frere et al. 1996; Millones et al. 2005; Morgenthaler et al. 2016; Barrionuevo et al. 2018).

Frere et al. (2008) found that rock shags living in sympatry with red-legged cormorants at Ría Deseado showed a different foraging behavior (feeding trip distance and duration) than in a colony from northern Patagonia where rock

shag breeds in allopatry (without red-legged cormorants). Despite the differences in the foraging patterns between these colonies, we found no notable difference in the diet of rock shags from Ría Deseado compared to that from northern Patagonia (Malacalza et al. 1997; Punta et al. 2003; Bulgarella et al. 2008; Sapoznikow and Quintana 2009). We found some taxonomic differences in the prey composition, likewise related to their distribution and availability, but no important ecological differences in prey types, indicating a consistent benthic diving behavior.

The comparison of the feeding behaviour of the rock shag and the red-legged cormorant at Ría Deseado colony yields little overlap in their feeding locations and shows that red-legged cormorants feed significantly closer to the colony and undertake shorter foraging trips than rock shags do (Frere et al. 2008). The diet of breeding red-legged cormorants, studied through a similar dietary combined framework, show a low diversity and a strong pelagic component (Morgenthaler et al. 2016). Patagonian sprats and Patagonian

squid are the two main prey (Morgenthaler et al. 2016). Therefore, the Patagonian squid seems to be the only important prey that both rock shags and red-legged cormorants are sharing, suggesting little trophic overall overlap. The interspecific differences in the feeding areas and the diet composition of these two species belonging to the same guild could be indicating a certain level of trophic resources partitioning. Furthermore, two more species belong to the Phalacrocoracidae guild resort to the Ría Deseado estuary for feeding: the neotropic cormorant (*Phalacrocorax brasilianus*) and the imperial shag (*Phalacrocorax atriceps*). Therefore, this estuary is one of the very few locations in the world with four sympatric species of shags and cormorants, and constitute an ideal location to study the trophic segregation within this guild. Further knowledge on the diet composition of these four species is necessary.

The combination of pellet and stable isotope analyses offered a complementary framework to study the diet of this shag, in a much more robust manner than deploying each technique separately. The pellet analysis provided comprehensive trophic information regarding the prey spectrum, as well as a prior set of information to incorporate in the mixing models. The stable isotope analysis of blood and an adequate isotopic prey sampling design, allow for the quantification of assimilated prey through mixing models (Phillips et al. 2014). The incorporation of previous dietary information in the mixing model, particularly when the isotopic values of the prey types are not different enough or their position in the bivariate space is not geometrically adequate like in our study help to reduce the credibility intervals and orientate the models towards the most appropriate dietary proportion combinations (Phillips et al. 2014).

The dietary results obtained through this study for the rock shag at Ría Deseado framework are in agreement with previous studies from locations situated further North along the Patagonian coast. This study confirms the importance of benthic prey, and particularly of benthic fish species, and the low inter-annual variability in the diet of the shag. These results coincide with the previous research in relation to the exploitation of slow moving, predictable, but not abundant and low-energy density prey (Sapoznikow and Quintana 2009). The stable isotope approach allowed for the detection of subtle differences between the diet of adults and chicks, like the incorporation of higher proportions of cephalopods, an energy-rich prey, in the diet of chick rock shags.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and handling of animals were followed.

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